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Isolation of *Salmonella* Sp. and *E. Coli* from Fermented Milk Product (*Nono*) in Kuje Area Council (Fct, Abuja, Nigeria) and their Antimicrobial Resistant Status

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ABSTRACT

This study was undertaken to assess the incidence of antimicrobial susceptibility status of *Salmonella* species and *Escherichia coli isolated* from a fermented cow milk product (Nono) in Kuje, FCT, Abuja. For the purpose of this study, Kuje was divided into; Kuje North (KN), Kuje East (KE), Kuje West (KW), Kuje Central KC) and Kuje South (KS). In number, a total of 154 fermented samples of the products were collected for bacterial isolation and identification from the regions using conventional bacteriological identification methods. The identified isolates were tested for their antimicrobial susceptibility status using four different types of antibiotics by disc diffusion method. The prevalence of Salmonella sp. was 10/154, while that of E. coli was 18/154. The presence of Salmonella isolates was higher in those milk samples products where nearby stream was the primary source of available water for all routine domestic use including milk processing (KE and KN) respectively. All the salmonella isolates were found to be susceptible to gentamicin but 91.3% to amoxicillin, 90.2% to sulphamethoxazole-trimethoprim and 88.9% to tetracycline. Three of the Salmonella isolates showed multiple drug resistance to two drugs. The findings from this research showed that the fermented dairy milk product (nono) obtained from local processing households in Kuje, FCT, Abuja was contaminated with public health important bacterial species; *Salmonella* species and E. coli. Also of utmost importance is the resistance of the pathogens to certain antimicrobial drugs which call for attention. Therefore, in order to ensure safe quality of fermented milk products are supplied to the FCT, the respective stakeholders engaged in this business need to be educated in basic hygienic practices and the implication of poor sanitation on human health

KEYWORDS: Salmonella sp., Antimicrobial susceptibility; Escherichia coli; Fermented Product; Nono; Kuje

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INTRODUCTION

Fermented milk is an important diet in many parts of Nigeria and the world at large. The rural populace depends so greatly on milk and its products for both as food and as well as source of employment especially the women who often add value to the products (Rozenberg, *et al*., 2016). Milk and milk products are highly nutritious as such they are the most important single diet balanced meal in nature because it contains almost all the ingredients needed for growth and development in the right proportion. Although milk is regarded as a sterile fluid when it is obtained directly from the udder (alveoli) of the animal, it is however highly prone to contamination and spoilage by different microorganisms as soon as it is exposed (WHO, 2015). The quality of the product is therefore often compromised due to degradative activity of the contaminants. This explains why milk does not stay long after delivery from the natural source especially in high ambient temperature environment. There are occurrence of high pathogenic bacteria and fungi counts in milk that not only do they degrade the products but also produce toxic metabolic materials during their growth and normal activities which posse serious health concerns to the consumers

(Farzanh *et al*., 2012). The main sources of microbial contaminations are either from the udder, exterior of the udder, from the containers, the handlers or from the air and grass beddings around the processing areas. Due to the nutritious nature of milk and milk products, it is generally in very high demands. This demand does not however always eliminate safe and high quality products which therefore placed a demand on the producers to seek for methods for the manufacture of products with non-compromising qualities void of pathogenic microorganisms (Mersha *et al*., 2010). Foodborne pathogens are dangerous microorganisms are often present in milk with unwholesome qualities and are transmitted from place to place through foods contaminated by bacteria, fungi, protozoans and viruses. Common bacteria associated with milk contaminations which include *Escherichia coli, listeria monocytogenes, salmonella, Campylobacter, brucella abortus, staphylococcus aureus, Bacillus cereus, Mycobacterium spp. And clostridium botulinum* (Pal *et al.,* 2016*)* The presence of these organisms are usually of public health concerns. The use of antibiotics in the treatment of diseases associated with these microorganisms have given rise to several complications of drug resistance in the public health system. The irrational use of drugs not only limited to the veterinary antibiotics but also in food producing chains of residues of edible tissues and products. Gastroenteritis due to food-borne disease is one of the most common illnesses in Nigeria, and it is a leading cause of death among people of all ages in the country and many other African countries (Radostits *et al*., 2016). Unfortunately, lack of awareness especially in among the rural folks and lack of surveillance of food-borne pathogens, poor hygienic conditions and the wide spread cultural practice of raw milk consumption all contributed to the degeneration of health system due to drug resistance situations. *Salmonella* specie and *Escherichia coli* are common foodborne pathogens of public concerns associated with the consumption of milk and milk products. There are relatively no organized reports are structured systems to indicate the distribution and mode of occurrence of multiple drug resistant strains of these organisms generally among milk consumers and producers in many parts of the developing countries.

Milk and milk products are highly consumed because of their rich nutrients. It is therefore very conducive for the transmission of many disease-causing microorganisms (Oloso *et al*., 2018). There is emergence of antibiotic-resistant foodborne bacteria associated with milk product among the populace which has turned into a serious growing issue in current health system. *Salmonella* spp. and *E. coli* can be transmitted through feces and milk from infected cattle and their environment. In the past few

years, *Salmonella* serotypes and *E. coli* have become resistant to frequently used antibiotics that increased the treatment cost in food animal production and also affected the human population. Milk and milk products are highly consumed in the study area, yet, there is lack of adequate surveillance data on the occurrence of antibiotic-resistant bacteria obtained from dairy livestock farming in the study area and the possible implications on human health (Tajbakhsh *et al*., 2012).

Antibiotic resistance is a global public health concern that necessitate definite studies to ensure that adequate knowledge is available to all the human factor that could be involved in the production and distribution of milk and milk products (Addis *et al.,* 2011). Antibiotic-resistant bacteria as the etiology of infection although have been expanding at an alarming rate, can be checked if adequate precautions are put in place to address the haphazard use of antibiotics therefore fostering knowledge of the imperative variables for the rise, selection, and spread of antibiotic-resistant organisms in the environment (Joseph *et al*., 2017). Therefore, there is need for studies about drug resistance among the consumers of the product believed to be an important vehicle for the foodborne pathogens that causes serious human health problems because, milk is an essential food material among both rich and poor populations of the Nigerian citizenry, Kuje people inclusive.

The present study therefore aimed at determining the antibiotic-resistance status of the bacteria; *E. coli* and *Salmonella* spp. in *Nono*, a locally fermented dairy milk product in Kuje area council, Federal capital Territory, Abuja, Nigeria and the possible public health significance.

MATERIALS AND METHODS

Study Area

The study was conducted in in Kuje area council, Federal capital Territory, Abuja, Nigeria, from November 2021 - January 2022. The wet season is oppressive and overcast, the dry season is humid and partly cloudy, and it is hot year round. The temperature typically varies from 62^0F to 94^0F. Nnamdi Azikiwe International Airport (ABV) is about 14.69km from Kuje. Intensive and semi-intensive cattle dairy farms with mixed breeds of exotic and cross breeds are managed at the outskirts of the community at the area. For the purpose of this study, Kuje was divided into; Kuje North (KN), Kuje East (KE), Kuje West (KW), Kuje South (KS) and Kuje Central KC) (Figure1).

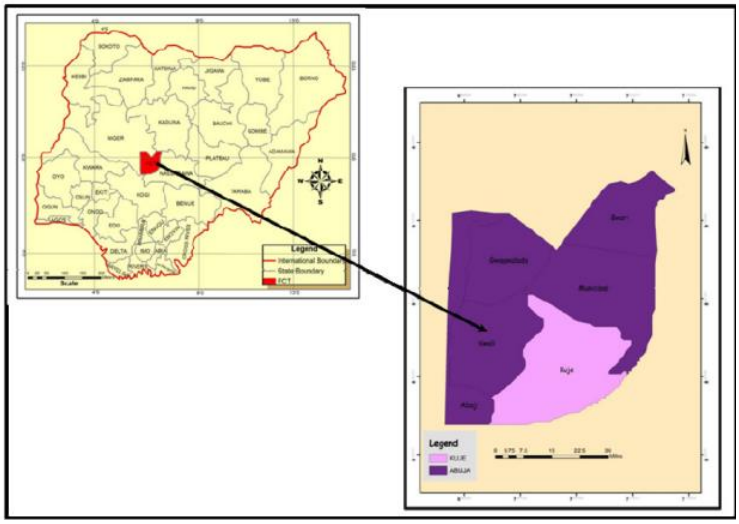

Figure 1: Location of the Study Area on the Nigeria Map

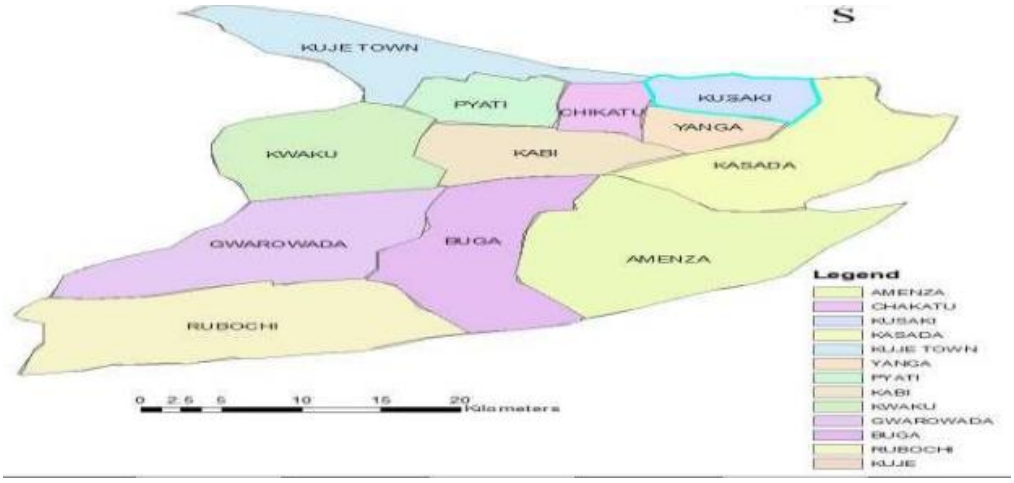

Figure 2. Map of Kuje Area Council showing the Various Wards

Study Design

In order to effectively carry out this study, a cross-sectional study was conducted to determine the antibiotic resistance status of *E. coli* and *Salmonella* species isolated from milk and milk products sold in the study areas.

Sampling Procedures

Milk samples were collected from individual dairy milk collectors and processors from Kuje Area.

Accordingly, majority of the farms were at the household/smallholder level, with small farm size and the products from such farms are usually small. The collection and processing of this products are at local, primitive levels at roadside and small market sites where the products are openly displayed. For the purpose of this study, simple random sampling technique was applied to collect raw and processed (spontaneously fermented products) samples from each group of collecting site and sale outlets. The milk and milk products were collected aseptically early in the morning time soon after milking and processing. Approximately about 4-5 ml samples were aseptically collected from and placed in sterile universal bottle by using sterile graduated pipette for each samples. the samples were labeled and immediately transported to the analytical laboratory (Thaker *et al*., 2012).

Isolation and identification of *E. coli*

After thorough homogenization, each milk samples were inoculated on MacConkey agar, and incubated at 37^0C for 24 hours. Discrete, typical colonies on MacConkey agar (pink, due to their ability to ferment lactose) were subjected to Gram staining procedures and observed for their staining and morphological characteristics. The positive results were then transferred to Eosin Methylene- Blue (EMB) agar. The colonies with green metallic sheen on EMB agar which is typical feature of *E. coli* were transferred to nutrient agar to be used for secondary biochemical tests (IMViC tests) (Thaker *et al*., 2012). A standard reference strain of *E. coli* was used as a quality control.

Isolation of *salmonella* species

For the *Salmonella* identification, the milk sample was homogenized and 1 ml was added to 9ml of sterilized buffered peptone water and incubated overnight at 37°C.

Then for selective enrichment, 0.1ml of pre-enrichment was transferred to 10 ml of Rappaport Vassiliadis Soya broth (RVS broth) and were then incubated at 41.5 ºC for 24 hrs. Each selective enrichment broth bottle was thoroughly shaken and then a loop full from each was streaked on sterile plates of Xylose Lysine Deoxycholate (XLD) agar and all plates were then aerobically incubated at 37°C for 24 hrs. Pink colonies with or without black centers were transferred to nutrient agar for further test. Then secondary biochemical tests (IMViC tests), TSI, urea and lysine were conducted. A standard reference strain of *Salmonella* was used as a quality control. After the incubation, suspected colonies were confirmed by using GEN III microplate, BIOLOG system (Harrigan and McCance. 2014).

Antimicrobial susceptibility test

The antimicrobial susceptibility test was performed following the standard agar disk diffusion method using commercial antimicrobial disks. The selection criteria of the antibiotics depended on the regular use of the antimicrobials in the animal and human treatments. Mueller-Hinton agar media was used for susceptibility testing. The isolated strains of bacteria were tested for sensitivity to commonly used antimicrobials in the area which includes; Gentamicin, Trimethoprim-sulfamethoxazole (SXT), Tetracycline (TE) and Amoxicillin. A standard reference strain of *E. coli* used as a quality control. Interpretation of results was made according to CLSI Guideline (CLSI, 2015).

RESULTS

Occurrence of *E. Coli* And *Salmonella* Species From Some Fermented Milk Samples In Kuje Area Council

In the study, a total of 154 milk samples were collected from dairy cattle farms and milk processors for the isolation and identification of *E. coli*, and *Salmonella* species. The relative occurrence of these pathogens are summarized in table 1. *E. coli* and *Salmonella* species were detected in 18/154 (11.7%) and 10/154 (6.5%) respectively.

Table 1. Occurrence of *E. coli* and *Salmonella* species.

Bacteria Strains	Positive milk samples (%)
E. coli	18/154 (11.7%)
Salmonella sp.	10/154 (6.5%)

The prevalence of *Salmonella sp*. was 18/154 (11.8%) distributed as KN (28%), KE (39%), KW (11%) and KC (22%) respectively, while that of *E. coli* was 5/154 (3.27%) in KN (40%), KE *Salmonella sp*. was 10/154 (11.8%) distributed as KN (28%), KE (39%), KW (11%) and KC (22%) respectively, while that of *E. coli* was 18/154 (3.27%) in KN (40%), KE (60%) and no occurrence in KW and KE.

Table 2. Distribution of Salmonella and *E. coli* Isolates in The Study Area

Source of Milk Sample	Number of Samples Tested	Positive Samples	No. of *E. coli*	No. of Salmonella sp.
KN	32	6	2	4
KE	45	9	4	5
KS	43	6	5	1
KW	10	3	3	0
KC	24	4	4	0

A total of Eighteen (18) *E. coli* and Ten (10) Salmonella isolates were tested for antibiotic susceptibility using four antimicrobial discs according to the CLSI guidelines. All *E. coli* isolates were found to be 100% susceptible to gentamicin followed by amoxicillin (95%) and sulphamethoxazole-trimthoprim (90%) and then tetracycline (76%) as shown in figure 1 below.

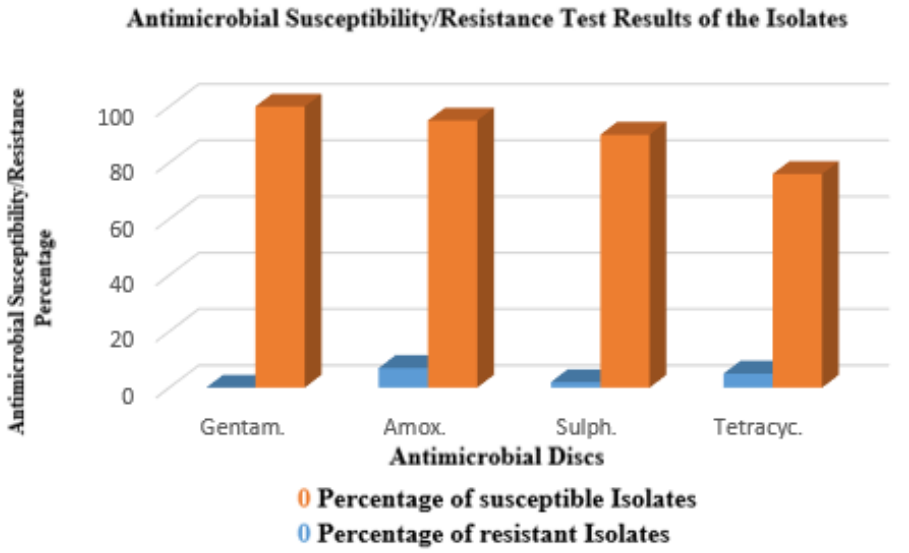

Figure 3. Antimicrobial susceptibility/Resistance Test Results of Isolates.

Two Salmonella isolates showed resistance to amoxicillin (7%) and one for sulphamethoxazole-trimethoprim (2%) and one to tetracycline (5%). Three of the isolated *E. coli* species showed multiple resistance to two drugs (one isolate to Amoxicillin-Tetracycline (5%) and the other to Tetracycline-Sulpha-Trimethoprim (7%)).

DISCUSSION

Milk and milk products are highly perishable products especially in the tropics where the environmental conditions are conducive for the growth and multiplication of spoilage microorganisms. The food items under study are nutritious for the wellbeing of human and have been fulfilling the roles of reducing hunger in many regions where the producing animals are found. *E. coli* is naturally found associated with the intestines of human and animals, therefore, their transmission from one place to another are easy where proper hygiene is not observed (Disassa *et al.*, 2017). This is therefore a global health concern due to the presence of toxigenic strains among the food pathogens that could cause gastro-intestinal disturbances and other life threatening syndromes on the consumer (Bedasa *et al*., 2018). Salmonella and *Escherichia coli* are regarded as both indicator of fecal contamination as well as indicators of poor hygiene and sanitary practices during milking and further handling at all processing stages. In our study, a total of 154 locally fermented milk samples were studied and from these, *E. coli* and *Salmonella* species were detected in 18/154 (11.7%) and 10/154 (6.5%) respectively. The isolated percentage of *E. coli* is in agreement with the report by (Mekuria *et al*., 2018). On the other hand, the present study was relatively lower as compared to the studies by (Lye *et al*. 2014).

Salmonella species because enteric infection characterized mainly by gastroenteritis on humans and other animals worldwide, and sometimes in severe cases it can result in systemic infection and even death. In general, *Salmonella* prevalence observed in study was 10/154 (6.5%).

It possible that the variation that was observed in prevalence of *E. coli* and *Salmonella* species in different studies may be due to difference in sample size, farming system, farm size, milking equipment, milking technique, geography, ecology, duration of milk transportation, and hygienic conditions especially the type and sources of water used (Abunna *et al.*, 2018). Although the occurrence of the isolated bacteria may not necessarily indicate a direct faecal contamination of milk, rather, it could be an indicator of poor hygiene and unsanitary practices during milking and processing of the product thereby presenting a potential hazard for the consumers (Ranjbar *et al*., 2018).

The occurrence of antimicrobial resistance among the isolates is of great health concerns. From the study, *E. coli* isolates were highly sensitive to the major antibiotics whereas salmonella species showed some levels of resistance as shown in Figure 3. Multiple drug resistance patterns were also observed. The presence of antimicrobial resistance could be due to overuse and misuse of the antimicrobials by herd owners and animal health professionals who are generally out to make money at the detriment of human lives. Drugs like tetracycline are commonly used in the country for treatment and disease prevention in both human and animal health management. There has been some direct correlation between the misuse of these drugs and high incidence of drug resistance (Ombarak *et al*., 2016).

CONCLUSION

From this study, we could see that fermented milk products sold in Kuje Area council of FCT, Nigeria are contaminated with poor hygiene indicator organisms that are life threatening owing to both their pathogenic as well as antimicrobial susceptibility status. The presence of these

organisms in the products could be as a results of contaminated udders, contaminated water, poor sanitation practices, contaminated containers, and even from milk handlers themselves. Some processors depend solely on stream water that are prone to faecal contaminations. The high ambient temperature in the study area could also be a good factor responsible for the occurrence of these microorganisms because it is conducive for them. Therefore, in order to ensure quality products from the milk, the various stakeholders in milk processing should be trained in the aspects of basic hygiene practises to ensure that the health of the populace is not compromised.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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